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## Newborn splenic volumes vary under different malaria endemic conditions

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**SUMMARY** Ultrasound was used to measure newborn splenic dimensions and calculate the volumes in a malarious and a non-malarious region of Papua New Guinea. The median splenic volume of infants born in Madang, where malaria transmission is high throughout the year, was 5.2 cm<sup>3</sup>/kg, while that of infants born in Goroka, where malaria is not endemic, was 2.6 cm<sup>3</sup>/kg. The cause of this difference is unknown, but possible explanations include fetal exposure to malaria antigens in utero and the high incidence of inherited red cell disorders in the malarious regions of Papua New Guinea.

In 1956 Bruce-Chwatt reported that the mean spleen weight of newborn African infants was one and a half times greater than that of European infants.<sup>1</sup> An association with holoendemic malaria was suggested, and as congenital malaria is rare, it was hypothesised that parasite antigens or specific fractions of maternal antibody could cross the placenta to cause stimulation and proliferation of the fetal spleen. More recently, the offspring of mice infected with *Plasmodium berghei* were shown to have increased spleen to body weight ratios compared with controls.<sup>2</sup> Splenic enlargement occurred without fetal parasitaemia, and the authors postulated that transplacental passage of soluble malaria antigens may occur.

These two studies, both using postmortem findings, suggest that fetal spleen size may be increased in association with maternal malaria. To our knowledge, there are no data on newborn spleen size in live infants born under different malaria endemic conditions. Clinical examination is inadequate for detecting mild degrees of splenomegaly, but recently ultrasound has been used successfully to estimate splenic volume in neonates.<sup>3</sup>

The aim of this study was to measure newborn splenic volumes in Papua New Guinea using ultrasound, and to compare results from a coastal region with year round malaria transmission with those from a highland region where malaria is not endemic. Cord sera were collected to measure concentrations of total IgM and malaria specific IgM, as an indicator of prenatal antigenic stimu-

lation, and passively acquired malaria specific IgG, as an indicator of maternal malaria experience. In Madang, cord and maternal haematological indices were also studied.

### Subjects and methods

#### SUBJECTS

Splenic volumes were measured on singleton infants born at 37-41 weeks' gestation at Madang General Hospital and Goroka Base Hospital. Gestational age was assessed by the method of Dubowitz *et al.*<sup>4</sup> Approval was obtained from the local ethical committee and the mother's permission was sought to measure the baby's spleen.

#### STUDY AREAS

Madang is on the north coast of Papua New Guinea in an area with a high level of malaria transmission throughout the year. The overall prevalence of malaria in rural Madang varies from 35% to 43% (all species, all ages) the contribution by each species being *P falciparum* 70%, *P vivax* 25%, and *P malariae* 5%.<sup>5</sup> In urban Madang, malaria incidence is lower due to decreased vector transmission and improved access to health services. Goroka is in the Eastern Highlands Province at an altitude of 1650 metres. Early studies found no evidence of malaria in this region, but it now occurs sporadically as a result of development and increased communication with coastal areas.<sup>6</sup>

## SPLENIC VOLUME MEASUREMENTS

The ultrasonic apparatus used in both hospitals was a Toshiba Sonolayergraph SAL-22A (Toshiba Corporation, Japan), which is a real time linear scanner with a 3.5 MHz transducer and distance caliper. The calibration was checked before starting the study. Ultrasonography was carried out within 24 hours of birth.

To estimate splenic volume, the infant was placed in the right lateral position, and the spleen visualised in the left upper quadrant. After adjustment to give the optically largest section area in the longitudinal and transverse plane, the organ was measured as length (L) and depth (D<sub>L</sub>), and breadth (B) and depth (D<sub>B</sub>). The splenic volume was calculated using the volume formula for an ellipsoid:

$$\text{Splenic volume} = \frac{0.523L \times B \times D_L + D_B}{2} \text{ (cm}^3\text{)}$$

## BLOOD SAMPLING AND LABORATORY PROCEDURES

Cord blood samples were obtained immediately after delivery of the placenta. Serum was separated by centrifugation, and stored at -40°C until analysed. Thick and thin blood films were prepared from maternal peripheral blood, placental blood, and cord blood. These were examined for malaria parasites after staining with Giemsa. Cord serum total IgM concentrations were measured by single radial immunodiffusion using commercial goat anti-serum to human IgM and a World Health Organisation reference serum.<sup>7</sup> The lower limit of detectability was 0.02 g/l. Malaria specific IgG and IgM concentrations were measured by an enzyme linked immunoadsorbent assay (ELISA) technique using a sonicate of *P. falciparum* infected red blood cells as the malaria antigen.<sup>8</sup>

In Madang, cord and maternal blood samples were also collected in tubes coated with edetic acid. The haemoglobin concentration was estimated by the cyanomethaemoglobin method, the haematocrit by a microhaematocrit method, and the mean corpuscular haemoglobin concentration was calculated.

## DATA FROM POSTMORTEM EXAMINATION

In order to obtain an estimation of normal spleen size, the spleen weights of 48 infants who were stillborn or died within 24 hours of delivery were recorded from the Sheffield Children's Hospital postmortem book. The specific gravity of splenic tissue is approximately one (by Archimedes principle), therefore the splenic weight gives an approximate estimation of splenic volume.

## STATISTICAL METHODS

The Student's *t* test was used for comparison of means, and the Mann-Whitney test for comparison of medians. Correlation coefficients were estimated by linear regression.

## Results

Splenic volume measurements were obtained from 20 infants in Madang and 41 infants in Goroka. The frequency distributions of splenic volume/kg birth weight are shown in the figure. The Goroka distribution is concentrated towards lower values compared with the Madang distribution, with little overlap. The median splenic volumes, lengths, breadths, and depths and interquartile ranges are shown in the table. The median splenic volume from Madang was double that from Goroka (Mann-Whitney test, *p*<0.0001). No infant had a palpable

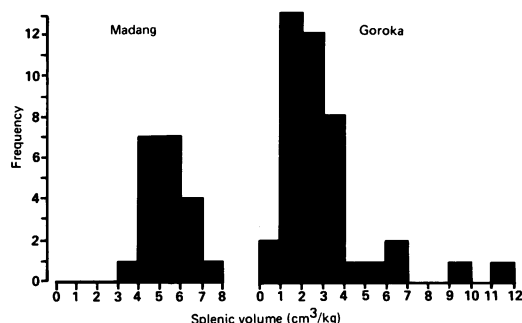


Figure Frequency distribution of splenic volume/kg birth weight.

Table Median splenic volume, length, breadth, and depth (interquartile range in parentheses)

Source	No of infants	Splenic volume (cm <sup>3</sup> )	Splenic volume/kg birth weight (cm <sup>3</sup> /kg)	Splenic length (mm)	Splenic breadth (mm)	Splenic depth (mm)
Madang*	20	14.5 (13.3-18.3)	5.2 (4.6-6.0)*	40 (36-45)	32 (29-35)	24 (21-25)
Goroka*	41	7.7 (4.3-10.8)	2.6 (1.5-3.5)	37 (32-42)	22 (17-30)	16 (14-21)
Sheffield†	48	6.5 (3.6-10.5)	2.8 (1.9-3.6)			

\*Mann-Whitney test, *p*<0.0001; †data from postmortem examination.

spleen nor clinical evidence of perinatal infection. Median splenic volume measurements did not differ in offspring born of primigravidae and multigravidae, and no difference was observed in values from neonates who were small for gestational age and appropriate for gestational age. The mean birth weight in Madang was 3050 g compared with 3120 g in Goroka.

Cord sera were collected from 20 infants in Madang, and 22 out of 41 infants in Goroka. Small amounts of IgM were detected in all samples. The median cord serum total IgM concentration from Madang was 0.045 g/l (interquartile range 0.034–0.057); this was not significantly different from that from Goroka: 0.052 g/l (interquartile range: 0.041–0.065). Splenic volume per kg body weight was negatively associated with cord serum total IgM concentration but the correlation was not significant in Madang or Goroka. All 20 cord sera from Madang were seropositive for malaria specific IgG, compared with two out of 22 cord sera from Goroka. No cord serum was positive for malaria specific IgM antibody. Maternal parasitaemia was found in two cases from Goroka, and five from Madang. No cord blood sample had demonstrable parasitaemia. The splenic volumes of the offspring of parasitised mothers were not significantly different from those of uninfected mothers.

In Madang, the mean (SD) cord haemoglobin concentration was 129 (16) g/l, the mean cord haematocrit was 44 (6.4)%, and the mean cord mean corpuscular haemoglobin concentration was 297 (19) g/l. The mean maternal haemoglobin concentration was 104 (22) g/l, the mean maternal haematocrit was 35 (4.5)%, and the mean maternal mean corpuscular haemoglobin concentration was 306 (27) g/l.

When the different haematological variables were compared with newborn splenic volume there was a significant positive correlation only for cord haemoglobin ( $r=0.45$ ,  $p<0.05$ ).

## Discussion

The main finding of this study was that newborns from a malarious coastal region of Papua New Guinea have significantly larger spleens than those from a non-malarious highland region. Comparison with British data from postmortem examinations suggests that the highland infants have normal spleens, whereas the coastal infants have subclinical splenomegaly.

Measurements in Goroka were made by DFM and in Madang by JAC. Circumstances made it impossible to assess interobserver variation, so we cannot entirely exclude the possibility that the

observed difference in splenic volumes was due to interobserver error. Identical techniques of measurement were used in the two centres, however, a clear outline of the spleen was obtained, and as splenic measurements from Madang were enlarged in every dimension compared with those from Goroka, it is likely that the observations are genuine.

In both hospitals it was routine practice to clamp the umbilical cord within 30 seconds of delivery, so variation in splenic volumes should not be due to differences in blood volume.

We do not know of any other data on spleen size in newborns in Papua New Guinea, but some early clinical studies are of interest. Metselaar reported that 75% of infants in a holoendemic malarious area of West New Guinea (now Irian Jaya) had a palpable spleen in the first three months of life.<sup>9</sup> Mackerras and Aberdeen also commented on the high number of large spleens in very young children on the north coast of Papua New Guinea, and suggested that 'many infants may be born with large spleens'.<sup>10</sup> Clinical splenomegaly was not found in newborns in the present study, even though the median splenic volume in Madang was double that in Goroka. It is generally accepted that the spleen must be twice normal in size to be palpable, but it is unclear whether this refers to length or volume.

The aetiology of newborn splenic enlargement in the coastal region is unclear. The fetal spleen has immunological and haematological functions, so mechanisms in both these categories should be considered. A recent study by Desowitz suggests that maternal malaria may cause stimulation of the fetal immune system.<sup>11</sup> He showed that the *P falciparum* specific blastogenic response of cord blood lymphocytes from infants born in Madang was 4.5 times greater than that of cord blood lymphocytes from infants born in Hawaii where malaria transmission does not occur. Possible mechanisms for immune stimulation include: (a) transplacental passage of soluble malaria antigens; (b) asymptomatic congenital malaria; (c) idioanti-idiotypic networking in the fetus after acquiring maternal anti-plasmodial IgG antibody; and (d) transfer of maternal lymphocytes and/or activated macrophages. Whether immune stimulation by any of these mechanisms would result in an increase in fetal spleen size is unknown.

In highly endemic malarious regions pregnant women are particularly susceptible to malaria because pregnancy appears to interfere with the immune response to the disease.<sup>12</sup> Five Madang women had parasitaemia at delivery, and all the Madang cord sera contained passively acquired malaria specific IgG, indicating a high degree of

maternal exposure to the disease. Splenic volumes did not differ in the offspring of parasitised and non-parasitised mothers, however, and the four Goroka cases with evidence of maternal malaria infection (two with maternal parasitaemia and two with passively acquired malaria specific IgG) did not have enlarged spleens.

Cord serum total IgM and malaria specific IgM concentrations were measured as a possible indicator of prenatal immune stimulation. No significant difference in total IgM concentration between Madang and Goroka was found, and malaria specific IgM was not detected; however, the high concentration of passively acquired malaria specific IgG in cord serum may interfere with the ELISA for malaria specific IgM. These data do not support the hypothesis that fetal immune stimulation is occurring, but it is possible that splenic enlargement could occur by immunological mechanisms independent of antibody production.

Fetal splenomegaly could also result from haematological mechanisms. Erythropoiesis in the spleen normally ceases before birth, but may resume in certain pathological conditions such as haemolytic anaemia.<sup>13</sup>

In Madang the mean cord haemoglobin concentration was 129 g/l, the mean cord haematocrit was 44%, and the mean cord mean corpuscular haemoglobin concentration was 297 g/l. These values are low in comparison with the normal ranges given for developed countries (cord haemoglobin 135–190 g/l, cord haematocrit 44–64%,<sup>14</sup> and cord mean corpuscular haemoglobin concentration 300–350 g/l<sup>15</sup>). Also a significant correlation was found between the cord haemoglobin concentration and the newborn splenic volume ( $r=0.45$ ,  $p<0.05$ ). It therefore seems plausible that the observed splenomegaly in Madang could be the result of a haematological disturbance leading to haemolysis or an increased need for erythropoiesis, or both. Possible causes for this include inherited red cell disorders and malaria.

The precise pathogenesis of malarial anaemia is uncertain, but it is thought to involve both defective production and increased destruction of red cells,<sup>16</sup> and it has been suggested that the immunological destruction of non-parasitised cells may occur.<sup>17</sup> Soluble malaria antigens may be non-specifically absorbed on to the surface of normal erythrocytes, and binding of antibodies to these antigens may trigger complement or cell dependent effector mechanisms resulting in removal of erythrocytes.<sup>18</sup> Placental transfer of soluble antigens and corresponding maternal antibodies could in this way cause fetal haemolysis and anaemia.

Several inherited red cell disorders are common in malarious areas of Papua New Guinea, particularly

$\alpha$  thalassaemia, ovalocytosis, and glucose-6-phosphate dehydrogenase deficiency. In the Eastern Highlands only 9% of individuals were found to have  $\alpha$  thalassaemia deletions,<sup>19</sup> but around Madang 97% have the genotype  $-\alpha/\alpha\alpha$  or  $-\alpha/-\alpha$ .<sup>20</sup> The incidence of high frequency hereditary ovalocytosis is between 11–30% in the Madang area but is rare in Highlands.<sup>21</sup> Glucose-6-phosphate dehydrogenase deficiency was found in 10–20% of males in Madang, and in 1% of Eastern Highlands males.<sup>20</sup> These inherited red cell disorders could cause a degree of anaemia in the newborn which might explain the increased splenic volumes found in coastal Papua New Guinea. A study in Polynesians on the influence of  $\alpha$  thalassaemia on haematological indices found that the mean cord haemoglobin from  $\alpha$  thalassaemia homozygotes (genotype  $-\alpha/-\alpha$ ) was 13 g/l less than that of normal individuals ( $p=0.01$ ), while that of  $\alpha$  thalassaemia heterozygotes (genotype  $-\alpha/\alpha\alpha$ ) was 4 g/l less ( $p<0.05$ ).<sup>22</sup> Whether  $\alpha$  thalassaemia alone would be sufficient to explain the anaemia and the increased splenic volumes found in Madang is uncertain. Another possibility is that fetal splenic enlargement could occur in association with maternal anaemia by the transplacental passage of a haemopoietic stimulus. Under these circumstances, however, high cord haemoglobin concentrations would be expected rather than the low concentrations found in this study.

Further work should be aimed at confirmation of these findings and differentiation between haematological and immunological causes of newborn splenic enlargement.

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